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Introduction

It is well known that plasma or serum levels of vitamin D and its metabolites reflect the nutritional status of vitamin D in humans.¹ In this analysis, the active forms of vitamin D, vitamin D₂ and D₃, are separated and analyzed using LC/MS/MS on the Varian 320-MS Triple Quadrupole mass spectrometer. LC/MS/MS is a very sensitive and accurate tool for vitamin D screening, and gives clear chromatographic peaks that could easily be applied to quantitative analysis in blood plasma or serum.

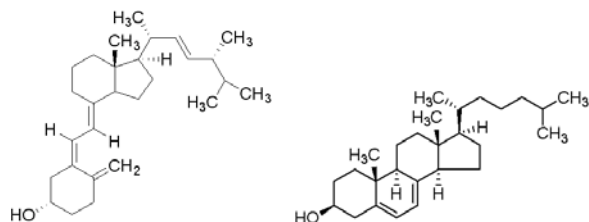


Figure 1. Chemical structure of vitamin D₂ (left) and vitamin D₃ (right).²

Standards of vitamin D₂ and D₃ were infused and the analytical conditions were optimized. Then, an injection at a concentration of 10 ppm was performed to yield MS/MS data for each of the analytes.

Instrumentation

- Varian 320-MS Triple Quadrupole LC/MS/MS with APCI source
- Varian 210-LC Binary Solvent Delivery Modules
- Varian Prostar™ 430 AutoSampler

Materials & Reagents

Vitamin D₂ and D₃ standards were purchased from Sigma (St. Louis, MO).

HPLC Conditions

Column: Polaris™ C18-A 3 μm, 100 x 2.0 mm ID
(Varian Part No. A2001100X020)

Solvent A: 50:50 Water:CH₃OH

Solvent B: Acetonitrile

LC Program:	Time (min:sec)	%A	%B	Flow (μL/min)
	00:00	100	0	400
	10:00	100	0	400

Injection Volume: 25 μL

Application Note 01511

Vitamin D₂ and D₃ Analysis on the Varian 320-MS Triple Quadrupole LC/MS/MS

MS Parameters

Ionization Mode:	APCI (positive)
API Drying Gas:	18 psi at 200 °C
API Nebulizing Gas:	55 psi
Vaporizer Gas:	18 psi at 100 °C
Needle:	5000 V
Corona Current:	2.00 μA
Shield:	600 V

Table 1. MS Segment Parameters.

Seg #	Analyte	Transition	Ret. Time	Collision Energy
1	Vitamin D ₂	389>270	4.01 min.	15.0 V
1	Vitamin D ₃	377>258	4.27 min.	15.0 V

Results & Discussion

Figure 2 shows the mass chromatogram of vitamin D₂ and D₃. This is MS/MS data using the precursor-product transitions listed in Table 1. This analysis was performed using an isocratic flow, so an LC gradient would undoubtedly provide increased chromatographic separation. However, the rapid analysis of multiple transitions is easily achieved on the Varian 320-MS, thus chromatographic separation is not required with a complex mixture of compounds.

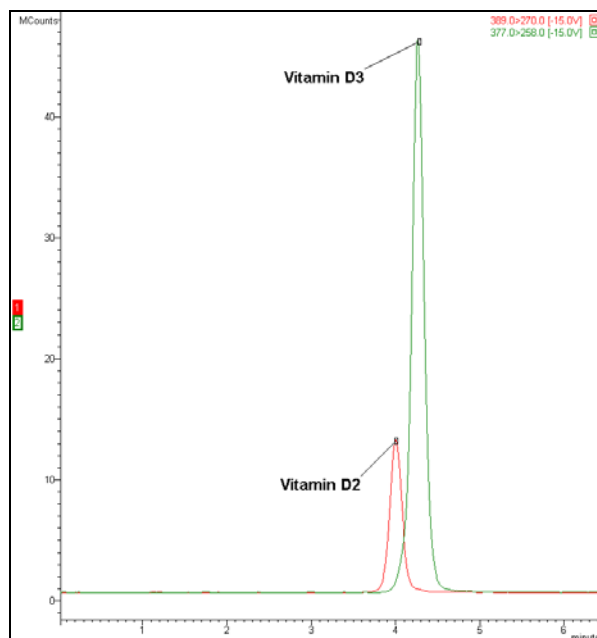


Figure 2. MS/MS chromatogram of Vitamin D₂ and D₃.

References

1. Kamao, M.; Tsugawa, N.; Suhara, Y.; Okano, T.;
"Determination of Fat-Soluble Vitamins in Human Plasma,
Breast Milk and Food Samples: Application in Nutrition
Survey for Establishment of 'Dietary Reference Intakes for
Japanese'." *Journal of Health Science* 2007, 53(3), 257-262.
2. Images from Sigma Aldrich website:
http://www.sigmaaldrich.com/Area_of_Interest/The_Americas/United_States.html. Accessed on March 3, 2008.

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